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Antitumor Efficacy of Seventeen Anticancer Drugs in Human Breast Cancer Xenograft (MX-1) Transplanted in Nude Mice

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Summary. *To validate the usefulness of a human tumor-nude mice xenograft system as a potential model in the secondary screening of anticancer agents, the antitumor activity of 17 anticancer drugs has been studied in the treatment of a human breast cancer tumor (MX-1) transplanted to nude mice. For evaluation of the antitumor activity of the drugs we employed the LD_{10} predetermined in BDF_1 mice as a standard therapeutic dose. Drugs were administered IV, IP, or PO, and antitumor activity was assessed by drug-induced growth inhibition measured by caliper. Among the 17 anticancer drugs, the most active compounds (maximum inhibition rate of tumor growth: ≥ 90%) are mitomycin C, chromomycin A_3, vincristine, vinblastine, vindesine, and hexamethylmelamine. Another group of compounds showed moderate activity (maximum inhibition rate of tumor growth: 89%–50%), these being adriamycin, daunomycin, mitoxantrone, bleomycin, 5-FU, 6-TG, and ftorafur. The remaining four drugs (peplomycin, cytosine arabinoside, 6-MP, and methotrexate) were inactive against the MX-1 tumor. These results suggested that in the nude mouse-human tumor xenograft system of the MX-1 tumor there was a good correlation between the antitumor activity of various anticancer drugs and their clinical efficacy; this system is therefore expected to be a useful model for the secondary screening system.*

Introduction

Successful transplantation of human tumors to nude mice with retention of their original function and morphological characteristics is possible [8, 9] and it is expected that the antitumor activity of anticancer drugs against human tumors transplanted into nude mice corresponds to the chemotherapeutic efficacy of human tumors. In our previous reports on alkylating agents [5, 6], the results indicated a reasonable correlation between clinical efficacy and antitumor activity against the human breast cancer designated MX-1, and thus the nude mouse-human tumor xenograft system is expected to be useful in the secondary screening of anticancer drugs. In the present study, the antitumor activity of seven anticancer antibiotics, six antimetabolites, three vinka alkaloids, and hexamethylmelamine was examined: the correlation of antitumor activity between xenograft and clinical efficacy of these drugs is discussed.

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Materials and Methods

Mice. Nude mice (nu/nu) on a BALB/c background, which had been bred and maintained under specific-pathogen-free conditions, were obtained from the Central Institute for Experimental Animals, Kawasaki, Japan, and were housed in autoclaved filter cap cages with autoclaved food and bedding. Cages were placed in a laminar-air-flow unit. Nude mice with a body weight of approximately 25 g and aged 6–7 weeks served as recipients. Five to six mice were used for each experimental group after randomization.

Tumor. The tumor was a human breast cancer designated MX-1 (Mammary Xenograft-1), diagnosed histologically as an infiltrating duct cell carcinoma (meduallary tubular carcinoma). It was established in nude mice in 1974 in the National Cancer Institute, USA, and it has been passaged by SC transplantation. The mass doubling time of the MX-1 tumor during the logarithmic phase was 3–4 days and the efficiency of transplantation was approximately 95%. The MX-1 tumor was estrogen-receptor-negative and the level of progesterone receptor was marginal.

Drugs and Treatment Schedules. The drugs, treatment schedule and sources are listed in Table 1. Most of the drugs were dissolved in physiological saline; ftorafur, hexamethylmelamine, and 6-MP, however, were suspended in 0.5% carboxymethylcellulose (CMC) (Wako Pure Chemical Ind., Tokyo, Japan). Solutions or suspensions of all drugs were prepared immediately before injection. Most drugs were given IV into the tail vein of mice; cytosine arabinoside, hexamethylmelamine, and 6-MP were given IP; and ftorafur was given PO. Control animals were given physiological saline or 0.5% CMC by the same route as the corresponding study preparation. We used the LD_{10} predetermined in BDF_1 mice as the standard therapeutic dose for 14 of the drugs used in this study, and this dose was decided on in light of the toxicological data of BDF_1 mice studied in our laboratory. In this study, the LD_{10} determined in BDF_1 mice, half and/or one-fourth of the LD_{10} were used. The dose levels of adriamycin and ftorafur were derived from the maximum tolerated dose (MTD) in nude mice (BALB/c, nu/nu), and that of hexamethylmelamine was selected on the basis of the chemotherapy study with nude mice in Battelle Columbus Laboratories, USA.

Evaluation of Antitumor Activity. The method of evaluation used in this study has been previously described [5, 6]. In brief,

Table 1. Drugs used in this study

Drug	Preparation	Route and schedule	Source
Adriamycin	PS[a]	IV × 1	Kyowa Hakko Kogyo Co., Tokyo, Japan
Bleomycin	PS	IV, q 4d × 3	Nippon Kayaku Co., Tokyo, Japan
Chromomycin A_3	PS	IV × 1	Takeda Chemical Industries, Osaka, Japan
Cytosine arabinoside	PS	IP, d 1–9	Nippon Shinyaku Co., Kyoto, Japan
Daunomycin	PS	IV × 1	Meiji Seika Co., Tokyo, Japan
5-Fluorouracil	PS	IV, q 4d × 3	Kyowa Hakko Kogyo Co., Tokyo, Japan
Ftorafur	CMC[b]	PO, q 4d × 3	Taiho Pharm. Co., Tokyo, Japan
Hexamethylmelamine	CMC	IP, q 4d × 3	Drug Liaison & Distribution Section, Division of Cancer Treatment, NCI, Bethesda, USA
Methotrexate	PS	IV, q 4d × 3 IP, d 1–9	Lederle (JAPAN), Tokyo, Japan
Mitomycin C	PS	IV × 1	Kyowa Hakko Kogyo Co., Tokyo, Japan
Mitoxantrone	PS	IV, q 4d × 3	Lederle (JAPAN), Tokyo, Japan
6-Mercaptopurine	CMC	IP, d 1–5	Sigma Chemical Company, Missouri, USA
Peplomycin	PS	IV, q 4d × 3	Nippon Kayaku Co., Tokyo, Japan
6-Thioguanine	PS	IV × 1	Drug Liaison & Distribution Section, Division of Cancer Treatment, NCI, Bethesda, USA
Vinblastine	PS	IV, q 4d × 3	Eli Lilly and Company, Indiana, USA
Vincristine	PS	IV, q 4d × 3	Eli Lilly and Company, Indiana, USA
Vindesine	PS	IV, q 4d × 3	Eli Lilly and Company, Indiana, USA

[a] Physiological saline
[b] 0.5% carboxymethylcellulose

a 2-mm tumor fragment was implanted SC into the back of each mouse. Treatment was initiated when the tumor mass reached about 150–200 mm^3 in volume. The length, width, and height of the subcutaneous tumors and the body weight of the tumor-bearing host mice were measured twice a week for 3 weeks. To evaluate antitumor activity, the maximum inhibition rate of tumor growth was calculated from the following formula:

$$1 - \frac{T_n}{T_0}\Big/\frac{C_n}{C_0} \times 100\,(\%)$$

where T_n/T_0 is the relative mean tumor volume in the treatment group between the initial day (0) and day n of treatment, and C_n/C_0 is that of the control group. The statistical significance of the difference in inhibition of tumor growth between experimental animals and controls was tested by Student's t-test.

Results

Among antitumor antibiotics, mitomycin C, chromomycin A_3 (toyomycin), adriamycin (doxorubicin), daunomycin (daunorubicin), mitoxantrone, bleomycin, and peplomycin were studied for antitumor activity against the MX-1 tumor. Mitomycin C, adriamycin, and chromomycin A_3 were administered by a single IV injection. The predetermined LD_{10} (IV) of mitomycin C in BDF_1 mice was 10 mg/kg, and single injections of mitomycin C at dose levels of 10 and 5 mg/kg were given, resulting in complete inhibition of tumor growth in all mice ($P \ll 0.001$). All five mice given 10 mg/kg and three of the five mice given 5 mg/kg were tumor-free for the 90 days of the observation period. Chromomycin A_3 was tested at doses of 1.4, 0.7, and 0.4 mg/kg. A chromomycin A_3 dose of 1.4 mg/kg, which is the LD_{10} (IV) in BDF_1 mice, effected 98% inhibition of tumor growth ($P \ll 0.001$). Doses of 0.7 and 0.4 mg/kg of this drug effected 38% and 18% inhibition of tumor growth, respectively.

The highest adriamycin dose of 15 mg/kg (MTD in BALB/c, nu/nu, IV) caused 74% inhibition of tumor growth ($P \ll 0.001$), whereas no significant inhibition of tumor growth was seen at doses of 7.5 and 3.8 mg/kg. Daunomycin was given in doses of 24 and 17 mg/kg as single IV injections. Half the animals treated with 24 mg/kg (LD_{10} in BDF_1 mice, IV) died, presumably due to toxicity. Daunomycin at a dose of 17 mg/kg induced 77% inhibition of tumor growth ($P \ll 0.001$).

Mitoxantrone, bleomycin, and peplomycin were administered IV every 4 days on 3 different days. Mitoxantone in doses of 3.1 mg/kg, which is the LD_{10} in BDF_1 mice (IV), and 1.6 mg/kg induced 76% ($P \ll 0.05$) and 39% inhibition of tumor growth, respectively. Bleomycin and peplomycin, which is an analog of bleomycin, showed low activity against the MX-1 tumor and the maximum inhibition of tumor growth obtained with these drugs was 59% ($P \ll 0.01$) and 34%, respectively. Among seven antitumor antibiotics tested in this study, mitomycin C and chromomycin A_3 produced significant inhibition of tumor growth in the MX-1 tumor, whereas adriamycin, daunomycin, mitoxantrone, and bleomycin showed moderate activity, and the activity of peplomycin was very modest.

Six antimetabolites, viz. 5-FU, 6-TG, ara-C, 6-MP, methotrexate, and ftorafur, were tested, but the maximum inhibition rates of tumor growth for these drugs were under 80%. Treatment with 5-FU or ftorafur every 4 days × 3 induced moderate activity, and the inhibition rates of tumor growth for these drugs were 75% and 66%, respectively. The highest dose of 6-TG, 20 mg/kg, was toxic and all mice died due to the toxicity; furthermore, the maximum inhibition rate of tumor growth for 6-TG was 55%. When the LD_{10} determined in BDF_1 mice was used, cytosine arabinoside induced slight inhibition (42%) of tumor growth, whereas 6-MP did not inhibit the growth of the tumor. The LD_{10} of

Table 2. Antitumor activity of 17 anticancer drugs against human breast cancer (MX-1) in nude mice

Drug	Dose of drug (mg/kg/dose)	Maximum inhibition of rate of tumor growth (%)[a]	Maximum loss of weight (%)
1. Antitumor antibiotics			
Mitomycin C	10	100***	16
	5	100***	–
Chromomycin A_3	1.4	98***	18
	0.7	38	–
	0.4	18	–
Adriamycin	15	74***	5
	7.5	46**	–
	3.8	39	2
Daunomycin	24	Toxic	22
	17	77***	29
Mitoxantrone	3.1	76*	31
	1.6	39	–
Bleomycin	80	59**	22
	40	43	5
Peplomycin	12	34	7
	6	5	1
2. Antimetabolites			
5-FU	52	75*	–
	26	Progression	–
6-TG	20	Toxic	–
	10	55	–
	5	26	–
Ara-C	49	42	–
	24.5	20	2
6-MP	62	Progression	6
	31	25	–
Methotrexate			
(q 4d × 3)	27	Progression	–
	13.5	Progression	–
(d 1–9)	1.8	21	–
	1.4	14	–
	0.9	Progression	–
Ftorafur	480	66*	2
	240	44	–
	120	29	–
3. Others			
Vincristine	1.2	100***	24
	0.6	98***	8
Vinblastine	5.7	Toxic	29
	2.9	100***	30
Vindesine	4	Toxic	25
	2	Toxic	27
	1	99***	–
	0.5	66	–
Hexamethylmelamine	200	99***	–
	100	31	–
	50	23	–

[a] Asterisks indicate significant differences from controls at $P < 0.001$ (***), $P < 0.01$ (**), and $P < 0.05$ (*)

methotrexate for BDF_1 mice is 27 mg/kg when given every 4 days × 3 (IV), and 1.8 mg/kg when given daily for 9 consecutive days (IP). These two treatment schedules induced no inhibition of tumor growth.

Three vinka alkaloids, vincristine, vinblastine, and vindesine, showed significant antitumor activity against the MX-1 tumor. Doses of 1.2 mg/kg per injection of vincristine and 2.9 mg/kg per injection of vinblastine every 4 days × 3 (IV) induced complete inhibition of tumor growth after 2 weeks from the start of chemotherapy. Doses of 4 and 2 mg/kg per injection of vindesine every 4 days × 3 (IV) proved toxic, and the majority of mice died 1 week after the treatment due to drug toxicity. Doses of 1 and 0.5 mg/kg per injection of vindesine (IV) induced 99% ($P < 0.01$) and 66% inhibition of

tumor growth, respectively. Two of six mice treated with 1 mg/kg were tumor-free for 90 days in the observation period. Hexamethylmelamine was tested at doses of 200, 100, and 50 mg/kg given by IP injection every 4 days × 3. At a dose of 200 mg/kg per injection of hexamethylmelamine 99% inhibition of tumor growth ($P < 0.001$) was induced, whereas doses of 100 and 50 mg/kg per injection were inactive.

The antitumor activity of 17 antitumor agents against human breast cancer (MX-1) transplanted in nude mice is summarized in Table 2. The most active compounds (maximum inhibition rate of tumor growth: ≥ 90%) are mitomycin C, chromomycin A_3, vincristine, vinblastine, vindesine, and hexamethylmelamine. The next most active drugs, with moderate antitumor activity (maximum inhibition rate of tumor growth: 89%–50%), were adriamycin, daunomycin, mitoxantrone, bleomycin, 5-FU, 6-TG, and ftorafur. The remaining four agents (peplomycin, cytosine arabinoside, 6-MP, and methotrexate) showed less than 49% tumor inhibition and were therefore considered to be inactive against the MX-1 tumor.

Discussion

We have already reported the antitumor activity of 17 alkylating agents against human breast cancer (MX-1) in nude mice [5, 6]. Among these drugs the most active compounds (with maximum inhibition rate of tumor growth: ≥ 90%) are cyclophosphamide, carbazilquinone, thio-TEPA, L-PAM, chlorambucil, dibromomannitol, and ACNU. Cyclophosphamide, carbazilquinone, thio-TEPA, L-PAM, and chlorambucil are clinically active against human breast cancer, and the overall response rates reported for these five drugs when used as single agents are 34%, 33%, 30%, 23%, and 20%, respectively. These data concerning the antitumor activity of alkylating agents suggested that the antitumor activity seen in the xenograft correlates well with clinical efficacy.

Among 17 anticancer drugs tested in this study, mitomycin C, chromomycin A_3, vincristine, vinblastine, vindesine, and hexamethylmelamine showed over 90% inhibition of tumor growth against the MX-1 tumor. Four antitumor antibiotics (adriamycin, daunomycin, mitoxantrone, and bleomycin) and three antimetabolites (5-FU, 6-TG, and ftorafur) showed moderate antitumor activity. The remaining four drugs (peplomycin, cytosine arabinoside, 6-MP, and methotrexate) were inactive. With regard to correlation between clinical response and antitumor activity studied in the MX-1 tumor, mitomycin C, vincristine, vinblastine, vindesine, and hexamethylmelamine, which were the most active preparations and showed over 90% inhibition of tumor growth in this study, are active against breast cancer clinically and the overall response rates reported for these five drugs are 37% [3], 20% [2], 20% [2], 29% [10], and 30% [1], respectively. For adriamycin, 5-FU, and methotrexate, however, there is a discrepancy between clinical response and the rate of tumor regression seen in this study. The overall response rates in clinical use of these drugs are reported to be 35% [2], 26% [2], and 34% [3], respectively, while with regard to antitumor activity against the MX-1 tumor these three drugs showed moderate activity. In particular, good antitumor activity was not obtained with methotrexate in spite of modifications of the treatment schedules used for this drug.

The chemosensitivity results using the nude mouse-human cancer xenograft system are influenced by the site of tumor transplantation and drug metabolism of mice. The highest level of adriamycin uptake in normal humans is seen in the liver, followed by lymph nodes, muscle, and bone marrow. Fat and skin show the lowest adriamycin uptake [7]. In this study the MX-1 tumor had been implanted in the back of nude mice, and we therefore surmise that high antitumor activity was not seen because the concentration of adriamycin at the back of nude mice is low. With regard to the low antitumor activity of 5-FU against the MX-1 tumor, the metabolism of 5-FU may be relevant. The metabolism of 5-FU in mouse is presumed to be faster than in human (T. Kobayashi, personal communication). Therefore higher doses of 5-FU are presumably required in mice to obtain antitumor activity equivalent to the clinical efficacy of 5-FU, and it may be necessary to maintain high blood concentrations of 5-FU in mice. Methotrexate also showed low antitumor activity against the MX-1 tumor. Administration every 4 days × 3 and on 9 consecutive days did not produce any significant antitumor activity, but we cannot explain the reason for the low chemosensitivity to methotrexate at this point.

In spite of the discrepancy between clinical efficacy and antitumor activity of some drugs, overall the chemosensitivity of the MX-1 tumor to various antitumor agents correlates well with the clinical efficacy of the agents. Availability of two or three breast cancer tumor cell lines in xenograft and of a different histological type from the MX-1 tumor would be a very useful aid to prediction of effectiveness and thus to selection of drugs to be used against human breast cancer. The earlier results suggested that the conventional screening system using P388, L1210 mouse leukemia, Lewis lung carcinoma, and B16 melanoma in mice was not suitable for the secondary screening [4, 11], and if the MX-1 tumor is utilized as one of a panel of human breast cancer tumors for this purpose it will allow the establishment of a more appropriate screening system for cancer chemotherapy.

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